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THE THYROID OF THE GUINEA-PIG IN EXPERIMENTAL DIPHTHERIC INTOXICATION *

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INTRODUCTION

Rupert Farrant[1] has reported the experimental production of changes in the thyroid gland of the guinea-pig similar to those observed in the human subject in diphtheria. He states:

> Firstly, the colloid becomes finely granular; secondly, it becomes vacuolated and partially absorbed; thirdly, an alteration occurs in the cells, they become more numerous, elongated, approaching the columnar, and arranged in masses; fourthly, the colloid becomes entirely absorbed and the walls of the vesicles become crenated and infolded; fifthly, the infolding and cell increase go on to transform the vesicles into solid masses of cells. These changes go on side by side so that several of them may be seen in the same section.

Farrant injected a number of guinea-pigs daily with the same dose of diphtheria toxin, which varied in strength from the minimal lethal dose to one-eighth part of it. Half the number of animals also were fed thyroid powder by mouth. These survived the animals not fed with this substance by one or two days. The doses of toxin were continued until the animals died, no mention being made of how soon after death the thyroids were removed to fixing fluids. He concludes that in the thyroid gland of the guinea-pig colloid absorption and cell reaction are produced by diphtheria toxin similar to the changes in the thyroid gland described in human diphtheria.

While working on thyroid problems, the writer was asked whether the changes described by Farrant could be produced in guinea-pigs by injections of diphtheria toxin given in such a way as to produce a course of toxemia similar to that in the human subject.

EXPERIMENTS

If we conceive that the initial toxin output in diphtheria in the human patient is not necessarily lethal, but that death is the consequence of a gradual overpowering of the defenses of the body, taking place in from one day or two, to a week or more, we should begin

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[1] Proc. Roy. Soc. Med., 1913, 6,[1] p. 21.

injections of toxin in doses considerably smaller than the lethal dose. With this in mind, sixteen guinea-pigs, varying in weight from 230 to 400 grams, were injected under aseptic precautions with an initial dose of 0.001 c.c. of a toxin, three weeks old, the minimal lethal dose of which was 0.007 c.c. The consecutive doses were varied in such a way as to simulate the course of diphtheric intoxication of varying severity (Table 1).

In order to avoid postmortem changes, the animals were watched closely, and as soon as they showed signs of oncoming death they were killed by bleeding from the heart, with the exception of Guinea-pigs 3, 5, 7, 8, and 12, which were killed at earlier periods. This was done with the possibility in mind that the repair of possible glandular changes might occur early in milder toxemias; that is, as soon as the system of internal secretions had adjusted itself to the circumstances. Therefore, animals were selected at random between the third and twelfth days of the course of injections in the hope of being able to demonstrate such lesions. In all cases a careful autopsy was made, the thyroids having been dissected out with all possible care so as to exclude traumatic changes. The thyroid itself at no time was handled, but was taken out with the surrounding connective tissue.

In a previous study of some thirty thyroids of apparently normal guinea-pigs, it was observed that the common fixing agents produce changes in the colloid which easily might lead to errors in the interpretation of colloid changes in an experimental study. To avoid this, one lobe of the thyroid was fixed for twenty-four hours in Zenker's fixing reagent without acetic acid, and the other in Bensley's osmic bichromate acetic acid mixture. The latter had given the best results in the preservation of colloid in the normal thyroids. After the fixed tissues had been washed in running water for several minutes, they were submitted to a very gradual dehydration in alcohols of increasing strength, twenty-four hours being allowed for each increase. The time for absolute alcohol was cut down to a few hours, as the latter tends to shrink the colloid. The tissues, after being passed through bergamot oil, bergamot oil-paraffin, and paraffin in the usual way, were embedded in 62 C. paraffin. Serial sections from 3 to 5 microns thick were stained by the neutral gentian method, safranin acid violet and fuchsin-methyl-green methods, as described by Bensley,[2] with Mallory's anilin blue connective tissue stain, and with hematoxylin and eosin.

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[2] Am. Jour. Anat., 1911, 17, p. 308.

TABLE 1

DAYS AND DOSES OF INJECTIONS

Weight of Guinea-Pigs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. 280 gm. .... Male	0.001	....	....	....	....	....	....	....	....	....	0.009	0.0105	0.021	....	Death 270 gm.			
2. 260 gm. .... Male	0.001	....	....	....	....	....	....	....	....	....	0.009	....	0.0105	....	0.021	....	....	Death 240 gm.
3. 280 gm. .... Female	0.001	....	....	Killed 280 gm.														
4. 340 gm. .... Male	0.001	....	....	0.001	....	....	0.0015	0.002	....	....	0.009	....	Death 330 gm.					
5. 330 gm. .... Pregnant female	0.001	....	....	0.001	Killed 320 gm.													
6. 340 gm. .... Pregnant female	0.001	....	....	0.001	....	....	0.0015	0.002	Death 320 gm.									
7. 300 gm. .... Male	0.001	....	....	0.001	....	....	Killed 300 gm.											
8. 270 gm. .... Male	0.001	....	....	0.001	....	....	0.0015	Killed 250 gm.										
9. 400 gm. .... Male	0.001	....	....	0.001	....	....	0.0015	0.002	....	....	0.009	....	0.014	....	0.021	Death 330 gm.		
10. 200 gm. .... Male	0.001	0.001	....	....	0.006	....	0.007	....	0.0105	....	....	Death 180 gm.						
11. 240 gm. .... Pregnant female	0.001	0.001	....	....	0.006	....	0.007	....	0.0105	....	....	Death 240 gm.						
12. 260 gm. .... Male	0.001	0.001	....	0.006	....	0.007	....	0.0105	....	....	....	Killed 240 gm.						
13. 270 gm. .... Male	0.001	0.002	....	....	0.002	....	0.014	....	0.021	....	Death 230 gm.							
14. 270 gm. .... Pregnant female	0.001	0.001	....	....	0.006	....	0.007	....	0.0105	....	....	....	Death 250 gm.					
15. 240 gm. .... Female	0.001	0.001	....	0.006	....	....	0.007	....	0.0105	....	....	Death 200 gm.						
16. 230 gm. .... Male	0.001	0.001	....	....	0.006	....	0.007	....	0.0105	....	....	....	Death 210 gm.					

In the thyroids of the normal guinea-pigs, fixed and stained in this way, it was found that vacuolation of the colloid is a common occurrence and is due to the fixing agents, as it is not seen in the fresh, unstained tissue. It was found further that the density of the colloid, as indicated by the staining intensity, varies to about the same degree and in the same proportion in the individual glands compared; also, that the size of the vesicles varies greatly in each individual gland, but that the compound glandular picture is always about the same in different guinea-pigs. As a general rule, the vesicles farthest away from the center of the gland are largest; but small vesicles are present also in this region. In the outer zone of vesicles the colloid appears to be more brittle, as indicated by the cracks made by the microtome knife.

It has occurred to me that the rather sudden and intense fixation of the outer vesicles leads to this phenomenon. The penetration of the fixing agents toward the more central vesicles is much slower and surely not as complete, and consequently the colloid preserves some of its flexibility.

"Langendorff-cells" are always found in the largest, commonly in the outer, vesicles, but they are present also in the central vesicles, only in less number.

Solid masses of cells, similar in structure to the cells of the vesicles (I shall call them "cell-buds" for short), are present in every normal gland, but they are scarce. At no time were the cells of these small masses found to undergo retrogressive changes. But neither were they found to be in an active state of hyperplasia, as indicated by the absence of mitotic figures, nor was there seen in these cells any colloid or its antecedent, as granules, which might point to a colloid secretion. The interpretation of these "cell-buds" is an open question. There are seen other very similar masses of cells which, to the unwary investigator who does not study serial sections, seem to be solid masses of cells. By following these through a series of sections, one learns that they are the top layers, so to speak, of vesicles. As the size of the individual vesicles varies greatly, it is easily understood that one may find many such "pseudo-cell-buds" at one level and few at another. Unless one makes a study of serial sections, this point will be overlooked, and if the mind of the investigator is in any way prejudiced in favor of hyperplasia of the gland a number of such "buds" in single sections will be interpreted wrongly.

Colloid was never found in the lymph spaces. With conventional stains, it is easy to mistake coagulated albuminous materials or densely agglutinated red blood corpuscles for colloid, as they stain very much the same as do the latter, especially when overstained. It is needless to say that a careful study of the section with a good apochromatic oil-immersion lens will eliminate this error. Of great help in this respect are the different stains mentioned. Mallory's anilin blue stain for connective tissue has given excellent results in differentiating colloid. It brings out the colloid in an intense greenish blue, while the albuminous materials stain faintly sky-blue, the white fibrous tissue dark blue, and the red corpuscles red in Zenker's fixation without acetic acid. Also, the acetic acid osmic bichromate mixture gives good results with this stain, except that the colors are not as brilliantly differentiated.

In the series of normal thyroids, invagination (infolding) of the walls of a few vesicles was seen in two glands. In all the others it was completely absent.

On the basis of these results the study was undertaken of the thyroids of the animals given diphtheria toxin.

In no instance was there found any demonstrable change in the colloid. In the thyroid of Guinea-pig 8 there were a few vesicles filled with an irregular coagulum instead of colloid. Vacuolation of the colloid was not increased as compared with that of the normal glands. Neither was there any visible tendency of the walls of the vesicles to break down, crenate, or infold. The solid masses of cells, or solid "cell buds," were in proportion to the normal. Mitoses were not found anywhere. An increase in the number of parenchyma-cells or their general changing to the elongated type, which to some extent is present also in the normal gland, could not be demonstrated. Instead of this, it was observed that the cells of Guinea-pigs 11 and 12 were larger and clearer than normal and that they seemed to bulge considerably toward their respective glandular lumina.

In Guinea-pigs 4 and 8, many vesicles presented one cell in the individual row which was about four times as large as the normal. The cytoplasm of these cells was very clear, the reticulum very fine, and the nucleus about twice the size of the normal one. These cells were usually flanked by one or two Langendorff-cells. Whether these gland cells are in a state of active secretion could not be proved, as no secretion granules could be demonstrated.

In Guinea-pigs 6, 11, 14, 15, and 16, the number of Langendorff-cells was increased over the normal. Along with this a marked edema and a slight round-cell infiltration were noticed. A slight edema together with a marked round-cell infiltration was seen in Guinea-pigs 7, 12, and 13.

Guinea-pigs 1, 2, 3, and 9 were entirely unchanged and 5 and 10 presented a slight round-cell infiltration alone. No colloid was seen in the lymph spaces.

DISCUSSION

If the results of this experimental study are compared with those of Farrant, the negative outcome of the writer's work is rather striking. As far as this work is concerned, postmortem changes were avoided entirely by taking out the glands from the freshly killed animal. The failure to observe early lesions was excluded by examining glands at different stages of the experiment. Traumatic changes were avoided by careful handling of the tissues concerned. Fixation artefacts were excluded by studying first a number of normal glands, fresh and fixed.

It may be argued that Farrant used larger doses early and repeated them oftener, and consequently obtained results which correspond in degree and kind to those found in severe human diphtheria. But he found such changes also when he used repeatedly small doses. In repeating his injections oftener, he did not allow the defense of the body to go into action. Undoubtedly, some such reaction occurs in human diphtheria. And as to the human material, we practically are confined to that from the severest and fatal toxemias. The changes in mild diphtheria have not been studied on account of lack of material.

The edema and round cell infiltration observed in those animals which were killed when dying seem to point to a vital reaction of the thyroid. But in most of these animals gross changes due to toxemia were seen in all the other organs also. Thus, the only sign of increased activity was perhaps the large, clear cells described. But, as they were found in only two glands of animals which had been given comparatively small doses, I do not think that we have the right to draw positive conclusions from this alone. Thus, the increase in Langendorff-cells in five glands is the only indication that there is some slight increase in thyroid reaction.

CONCLUSION

Experimental diphtheric toxemia produced in guinea-pigs in such a way as to simulate the course of human diphtheric toxemia does not lead to changes in the thyroid gland to the same degree as has been observed in the human thyroid gland in fatal diphtheria.

The changes observed in the thyroid of the guinea-pig are not sufficiently marked nor so uniformly parenchymatous as to allow us to conclude that this gland has a detoxicating action in experimental diphtheric toxemia.